

## Isocratic Analysis of Tetracycline Antibiotics Using a SUPELCOSIL LC-18-DB HPLC Column

*Tetracycline antibiotics have been difficult to separate using traditional methods. A specially-deactivated SUPELCOSIL HPLC column can eliminate these complications, and provide good data. The procedure described below is faster and less expensive than other methods, and the resulting chromatography is more symmetrical.*

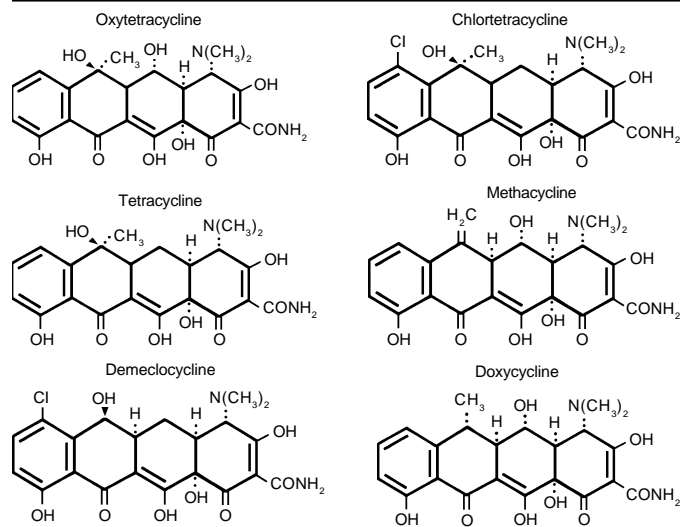
### Key Words:

• antibiotics • HPLC • SUPELCOSIL columns • tetracycline

Because tetracycline antibiotics have similar molecular structures (Figure A), they have always been difficult to separate by liquid chromatography (also GC). Furthermore, these antibiotics form hydrogen bonds with active sites on the silica surface of most HPLC columns. Broad and tailing peaks result, making quantification difficult. Sample recovery is poor and the chromatography irreproducible.

Several HPLC systems overcome these problems, to some degree, by using elaborate gradients, complicated solvent systems, or corrosive mobile phases with high salt concentrations (1,2). However, you also can separate these basic drugs, and other nitrogen-containing compounds, with specially deactivated SUPELCOSIL™ DB columns.

### Figure A. Tetracycline Antibiotics Have Similar Molecular Structure



797-0481, 0476, 0475, 0473, 0477, 0474

Using a 25cm SUPELCOSIL LC-18-DB column, and the mobile phase and conditions listed in Figure B, we resolved six commercial tetracycline drugs in 15 minutes. A 25cm column was needed to adequately resolve oxytetracycline from tetracycline. The ACN/THF/buffer ternary mobile phase provided adequate resolution and symmetric peaks. A binary THF/buffer mobile phase also resolved the six drugs, but with broad peaks. Oxytetracycline and tetracycline coeluted in an ACN/buffer mobile phase. The low pH provided the most symmetric methacycline and doxycycline peaks. At high pHs, these peaks tailed. Within the pH range of 4-8, tetracycline can epimerize to 4-epitetracycline (3).

### Figure B. A Deactivated HPLC Column Separates Tetracycline Within 15 Minutes

Column: SUPELCOSIL LC-18-DB (5-8355-U), 25cm x 4.6mm, 5µm packing, with Supelguard LC-18-DB guard column (kit, 59555), 2cm x 4.6mm, 5µm packing

Temp.: 35°C

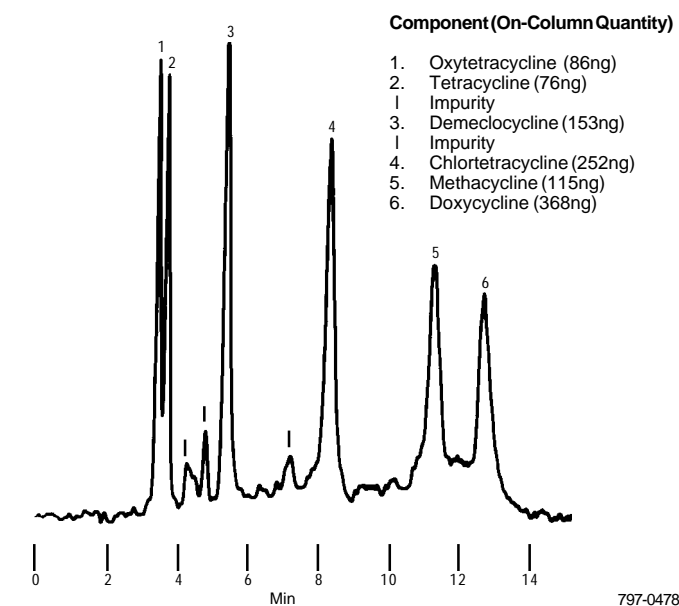
Mobile Phase: tetrahydrofuran:acetonitrile:0.005M Na<sub>2</sub>HPO<sub>4</sub> (pH to 2.0 with H<sub>3</sub>PO<sub>4</sub>) (9:9:82)

Flow Rate: 2mL/min.

Pressure: 2100psig

Det.: 254nm UV, 0.02 AUFS

Sample: 10µL mobile phase, component quantities listed on figure



A practical analytical procedure for tetracyclines must be adaptable. You should be able to use it to evaluate product quality (i.e., tablets) and monitor clinical samples (i.e., serum extracts). Furthermore, the method must separate tetracyclines well enough to allow detection of cross-contamination of products. Although microbiological (4,5), fluorometric (6-9), and paper chromatographic analyses have been used to monitor these drugs, these three methods lack selectivity. Tetracyclines' insolubility in nonpolar organic solvents makes normal phase liquid chromatography impractical. Other reversed phase HPLC methods for this analysis (1,2) are hampered by broad or severely tailing peaks, poor resolution, and long retention times. Frequently EDTA must be added to the mobile phase to improve sample recovery.

The analysis described here shows that a deactivated SUPELCOSIL LC-18-DB column will eliminate most problems encountered in other HPLC analyses of tetracyclines. The symmetric peaks enable an analyst to easily quantify these drugs. The procedure is faster and considerably less expensive than other means of analyzing tetracyclines. We recommend using a 2cm Supelguard™ LC-18-DB guard column to protect the analytical column from sample contaminants.

The packings in SUPELCOSIL DB columns incorporate silica specially treated to reduce the degree of ion exchange and improve the peak shape for basic compounds and other nitrogen-containing compounds. Thus, the same column can be used for additional drug analyses, minimizing column changes.

#### References

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#### Ordering Information:

SUPELCOSIL LC-18-DB Column  
25cm x 4.6mm ID **58355-U**

Supelguard LC-18-DB Kit  
Includes a 2cm x 4.6mm ID column, column holder,  
and connecting hardware **59555**

#### Trademarks

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